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Prediction of Substrate Removal Rates of Attached Microorganisms and of Relative Contributions of Attached and Suspended Communities at Field Sites

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16. ABSTRACT
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Prediction of Substrate Removal Rates of Attached Microorganisms and of Relative Contributions of Attached and Suspended Communities at Field Sites

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A mathematical model composed of a direct proportionality relationship between bulk water velocities and field-determined second-order microbial transformation rate coefficients, and the relative rate coefficient of a benchmark chemical, was developed for estimating the substrate removal rates of rapidly degraded chemicals by attached organisms in shallow (<1 m deep) aquatic ecosystems. Data from 31 field experiments involving the addition of 2,4-dichlorophenoxyacetic acid methyl ester (2,4-DME) in nine field areas were used to determine a field-derived second-order rate coefficient for microbial transformation of the ester. By using 2,4-DME as a benchmark chemical, the model was used to predict microbial transformation rates of the butoxyethyl ester of 2,4-dichlorophenoxyacetic acid (2,4-DBE) at five other field sites. The predicted half-lives of 2,4-DBE varied 1,500-fold and were within about a threefold range or less of the measured half-lives. Under conditions of mass transport limitation, the contributions of attached microorganisms relative to total microbial activities at various field sites were related to the ratio of water velocity, U, and depth, D, showing that historical definitions of ecosystems according to flow and depth characteristics are also valid for describing the process-related structure of ecosystems. An equation was developed for predicting the relative contributions of attached and suspended communities with values of U and D for lotic and lentic ecosystems. On the basis of this equation, attached microorganisms were expected to be insignificant in deep lentic ecosystems and suspended microorganisms were expected to be insignificant in shallow lotic systems for the same process carried out by both populations. Neglecting epiphytic microorganisms, both suspended and attached organisms were expected to be significant in wetlands.

For mathematical modeling of organic substrate removal rates in the aquatic environment, a suitable approach for predicting rates associated with suspended and attached organisms and their relative contributions in the system is needed. Most kinetic models that incorporate substrate removal rates have involved only suspended populations (2, 11, 14, 15, 18). Some research has been done on attached organisms (13, 17; C. J. Gantzer III, H. P. Kollig, B. E. Rittman, and D. L. Lewis, Water Res., in press), and some work also has been done to account for both attached and suspended organisms (7; C. J. Gantzer III, Ph.D. dissertation, University of Illinois, Urbana, 1986).

Rates of organic substrate removal in natural aquatic systems are governed by complex interactions of physical, chemical, and biological processes. These various processes have been elaborately addressed in the field of environmental engineering; however, it was not the purpose of this research to develop a model capable of using a wealth of physical, chemical, and biological data to accurately predict substrate removal rates. Instead, we were interested in how accurately organic substrate removal rates could be predicted with a very minimum of site-related information, such as water depth, bulk velocity, and degradation rate relative to a benchmark chemical. Even the inclusion of Froude (5) or Reynolds numbers (21) may not be appropriate for a non-site-specific model designed for making screening-level decisions concerning the anticipated contribution of microbial activities to the abatement of a myriad of organic chemical pollutants. For regulatory purposes, a non-site-specific predictive model that is accurate within a 10-fold range can be very useful.

For both suspended and attached microorganisms, many of the difficulties inherent in biological models have pertained to measurements of biomass concentrations and activities. In our research, we have found that removal rates of chemicals rapidly degraded by attached communities correlate well with the external biofilm surface, without considering biofilm volume. The consistency of chemical uptake rates per unit of external biofilm surface area (12) has allowed us to develop equation 1 for a second-order rate coefficient, k_A. This expression is applicable to rapidly utilized, hydrophilic chemicals and is based on water volume, V, colonized surface area, A, and a pseudo-first-order transformation rate coefficient, k_1, calculated from the rate of loss of parent compound from solution or the appearance rate of a metabolite (13). The apparent transformation rate, therefore, is k_1 [S], where [S] is chemical concentration.

$$k_A = k_1/AV^{-1} \quad (1)$$

The reliability of this second-order model has been shown, in that k_A obtained by using field-collected and microcosm-developed periphyton varied less than fivefold, even though biomass concentrations varied 100-fold or more (10).

Consequently, to estimate substrate removal rates, problems associated with biomass measurements may be circumvented for chemicals rapidly removed by attached communities. Mass transport of substrates to sites of biological activity, however, presents quantitative problems. Because of substrate depletion near cell surfaces, removal rates of chemicals rapidly utilized by attached organisms may be mass transport limited; hence, rates are determined by water

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turbulence near the biofilm surface (13). Limitations in mass transport also can occur with suspended organisms: however, major resistances to mass transport may be overcome for suspended organisms at very low water turbulences (3, 4, 16).

For shallow aquatic systems, we assumed that water turbulence near biofilm surfaces was sufficiently related to bulk water velocities, U, so that a measure of U would provide a satisfactory prediction of biofilm-associated substrate removal rates. We were not certain, however, whether variations in the shapes of the sides and the bottoms of lotic systems and the presence of colonized objects, such as rocks, would render this simple approach useless. One of the primary objectives of our work, therefore, was to determine whether U for shallow aquatic ecosystems at field sites could be used satisfactorily to predict substrate removal rates based on a relationship between U and k_A. We did not assume that the contours and roughness of the streambed were insignificant, only that field systems, such as streams or shallow rivers, are similar enough that k_A determined empirically at field sites could be used, along with U, to predict substrate removal rates at least within a 10-fold range of observed rates.

In predicting the relative contributions of attached and suspended communities in field ecosystems, we hoped that the relative contributions would be consistent, at least among similar systems for a given water depth, D. By determining the relative contributions of attached and suspended communities in various systems, we sought to develop an empirical relationship for predicting the relative contributions among systems that exhibit, on the basis of our data, similar ratios of attached-to-suspended organism activities.

MATERIALS AND METHODS

Reagents and analytical procedures. 2,4-Dichlorophenoxyacetic acid methyl ester (2,4-DME) was prepared by methylating 2,4-dichlorophenoxyacetic acid (2,4-D) with methanol, extracting the ester with methylene chloride, and evaporating the solvent. Gas-liquid chromatographic analysis of the product revealed no significant impurities. The butoxyethyl ester of 2,4-D (2,4-DBE) at 99.8% purity was obtained from Union Carbide Corp., New York, N.Y. Determinations of 2,4-D ester concentrations in isooctane extracts of water samples were made with a Tracor 222 gas chromatograph (Southern Analytical Inc., Oakwood, Ga.) equipped with a Ni-63 detector and a 2-m (4-mm inner diameter) column of 3% OV-1, 80/100-mesh Supelcoport. Rhodamine WT was obtained from the Environmental Services Division, Region IV, U.S. Environmental Protection Agency, Athens, Ga. Dye concentrations were determined at 554 nm with an Ultraspec 4050 spectrophotometer (LKB Instruments, Inc., Rockville, Md.).

Kinetic experiments. Pseudo-first-order substrate removal rate coefficients, k_1, were based on the loss of parent compounds from solution. In past studies (13), 2,4-DBE was quantitatively degraded to 2,4-D at rates equal to the loss of parent compound from solution. Rate coefficients, therefore, represented uptake and transformation rates.

Transformation rate coefficients were determined at field sites for total community (attached and suspended) activities by adding an aqueous stock of 2,4-D ester and rhodamine WT, a biologically inert dye, as in earlier studies (8). Values of k_1 were calculated from the natural logarithms of ratios of 2,4-D ester to dye concentrations versus time (Fig. 1). The dye served to account for dispersion, and we assumed that dispersion rates were the same for the dye and 2,4-D esters. Field concentrations of 2,4-D esters were less than 100 μg liter^{-1}. Grab samples were removed periodically as the dye and ester dispersed through the system and were immediately extracted with isooctane. Water velocities were determined by measuring the distance that the front of the pesticide-dye slug moved per unit time.

In lentic systems, water velocity was estimated on the basis of the greatest distance that the pesticide-dye slug moved in any direction from the point at which the slug was added. This procedure did not account for back-and-forth movements of the slug caused by wave or wind motion. Had this additional movement been taken into account, the two Okefenokee sites, for example, would have had water velocities about 20 times larger than those reported. Measurements of back-and-forth movements were difficult to obtain with acceptable accuracy; therefore, these measurements were not routinely made.

For laboratory determinations of the relative rate coefficients for transformation of 2,4-D ester, colonized Teflon strips were used according to published procedures (13). An aqueous mixture of the methyl and butoxyethyl esters was added to the microbial preparations, and relative rate coefficients were calculated by dividing the k_1 values of 2,4-DBE by the k_1 values of 2,4-DME. Experiments were also done, showing that mixing the esters did not affect their respective transformation rates.

Second-order substrate removal rate coefficients for attached populations, k_A, were based on a ratio of biofilm-colonized surface area to container water volume in laboratory reactors and on a ratio of colonized surface area per unit volume of water at field sites. As in other studies (13), k_A determinations in the laboratory involved the use of paddle-stirred reactors and Teflon strips that were colonized with periphyton for approximately 6 weeks. To test the effects of colonization time, some strips were colonized for up to 1 year in a local municipal water treatment facility. To preclude significant contributions of suspended organisms sheared from biofilms, reactors were stirred for several minutes, the paddle velocities were lowered about 90%, and the water containing sheared organisms was replaced before k_A values were determined. Experiments designed to test the effects of water velocity on k_A were similarly treated, and substrate removal rates were measured from the highest velocity to the lowest.

In addition to colonized Teflon surfaces, naturally colonized rocks were collected from two local streams (Harris Shoals Park and Watson Mill Bridge State Park) and one river (Oconee River). Flat rocks were selected that fit into the laboratory reactors in a manner similar to Teflon strips. Field ratios of A/V were determined from average water volumes per square meter of bottom surface area, typically using 10 to 50 depth measurements, depending on the area covered by the dye solution added to the site. Streams were shallow and varied greatly in bulk water velocity, with U/D values from 190 to 64,000 h^{-1}. Lentic systems were poorly mixed; therefore, we sampled shallow areas (approximately 0.1 m deep) and dipped grab-sample containers (1-liter beakers) down to the sediment surface. This manner of sampling created momentary turbulence and probably produced somewhat higher rate coefficients than would be obtained in undisturbed systems.

Values of k_1 for suspended organisms were determined from flask samples containing about 1 liter of water from the field site. Water samples from lotic systems were shaken on

an incubator shaker at temperatures approximating those of the field site. Water samples from lentic systems were allowed to float at the site to react at representative temperatures. No differences in substrate removal rates could be detected among suspended-organism samples either when rapidly shaken or stationary; therefore, mixing rates of suspended populations were not considered significant.

These procedures yielded independent determinations of substrate removal rate coefficients, based on ratios of 2,4-D ester to dye concentrations (Fig. 1).

Field sites. At 20 field sites, 31 kinetic experiments were done with 2,4-DME. Some locations were different sites of the same field area. Also, some experiments were done as replicate experiments at the same site. A total of nine field areas located in the southeastern United States were studied. Data from these sites were used to calculate a field-derived second-order microbial transformation rate coefficient for 2,4-DME.

The diversity of the sites is indicated in the following brief descriptions: Chalet Pond, a several-acre pond with an algae-covered mud bottom; Corkscrew Swamp, a peat-bottom swamp; Elders Covered Bridge Park, a granite-bottom stream covered with a very thin biofilm and with some sand-bottom areas; Harris Shoals Park, a granite-bottom stream with a filamentous biofilm; Iron Creek, a sand-bottom stream with a flocculant bacterial biofilm; Okefenokee Swamp, a peat-bottom swamp; Oconee River, a sand-bottom river approximately half covered with grass-like aquatic plants and filamentous algae; Mountain Creek, a stream with a gravel-bottom reach with little visible periphyton, a cobble-bottom reach (approximately 10-cm-diameter stones) with no visible periphyton, and a cobble- and sand-bottom reach with no visible periphyton; and Watson Mill Bridge State Park, a periphyton-covered, granite-bottom stream. All of the sites were studied during the summer and fall of 1986 and 1987, when water temperatures ranged from approximately 20 to 25°C, except for a stagnant, shallow area (site 5) in Watson Mill Bridge State Park, which had a temperature of 32°C.

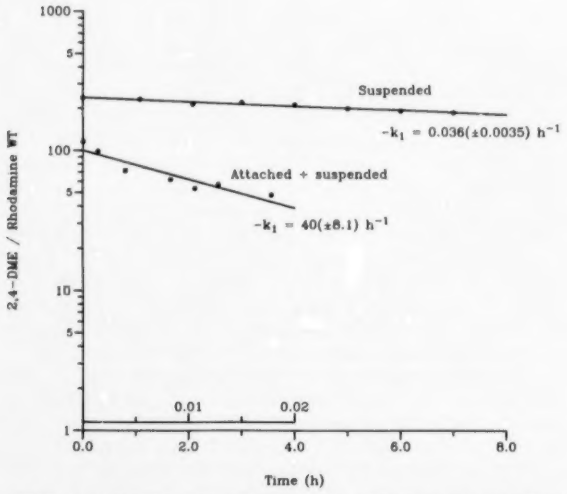

FIG. 1. Substrate removal rates of 2,4-DME were determined independently for total (attached and suspended) and suspended organisms in the field by adding a mixture of 2,4-DME and rhodamine WT to the aquatic systems and water column samples taken at the sites.

At five additional field sites, 2,4-DBE half-lives were measured and compared with predicted values by using a second-order transformation rate coefficient of the benchmark chemical, 2,4-DME. These sites included High Shoals, a granite-bottom stream completely covered with periphyton; Skidaway Creek, a sand-bottom stream partially covered with a filamentous biofilm; Corkscrew Pump Station no. 1, a peat-bottom swamp; Orange River, a sand-bottom stream completely covered with periphyton; and Okefenokee Canal, a peat-bottom swamp.

RESULTS AND DISCUSSION

With data from the 31 field experiments (Fig. 2), 2,4-DME removal rates appeared to satisfactorily conform to a direct proportionality relationship between bulk water velocities, U, and second-order rate coefficients, k_A. Although the data did not represent a perfectly linear function, nonlinearity probably resulted largely from underestimating water velocities in lentic and low-velocity lotic systems, as noted in the description of our kinetic experiments. Moreover, the accuracy of the model in predicting 2,4-DBE half-lives, discussed later, suggested that a direct proportionality relationship adequately described the relationship between k_A and U for the purposes of our model. Because the range of velocities included, on one extreme, lentic systems, such as swamps and ponds, and on the other extreme, very rapidly flowing streams, this result was very encouraging.

Five replicate k_A values determined on the same day at site 4 in Watson Mill Bridge State Park had a mean and standard error (SE) of 23,000 ± 300 liters m^{-2} h^{-1}, representing a coefficient of variation of only 5.8%. Five replicate values at site 2 of Elders Covered Bridge Park, however, gave a mean and SE of 3,800 ± 1,500 liters m^{-2} h^{-1}, representing a coefficient of variation of 87%. An almost 10-fold variation in some of the data, therefore, can be explained as experimental error involved in determining k_A in the field.

Two k_A values, from site 3 in Elders Covered Bridge Park and site 3 of Watson Mill Bridge State Park, exceeded a 10-fold departure from what the direct proportionality relationship predicted, indicating a spatially related depression of 2,4-DME removal rates. No removal was detected at site 3 of Elders Covered Bridge Park within the exposure time tested (therefore, this datum could not be shown in Fig. 2), and the k_A for site 3 of Watson Mill Bridge State Park was about 40 times lower than the predicted value. When these sites were tested about a month later, the new k_A values fell well within a 10-fold departure from predicted values.

Values of k_A determined from laboratory-colonized Teflon strips were equal to values obtained with naturally colonized rocks collected from one field site and were approximately one-half the values obtained with rocks from two other field sites. Teflon strips colonized in the field for 1 year also yielded k_A values that were double the values obtained with strips colonized for approximately 6 weeks in laboratory microcosms. Differences in microbial community structure and biomass concentrations account for only about a fivefold variation in k_A, and no significant differences between laboratory and field-collected microbiota exist for several rapidly degraded chemicals we have studied (10, 12). On the basis of these results, we concluded that rate data obtained with laboratory microcosms could be satisfactorily used for estimating substrate removal rates at field sites.

The consistency of k_A among diversely populated communities indicates that typical, mature biofilm surfaces are

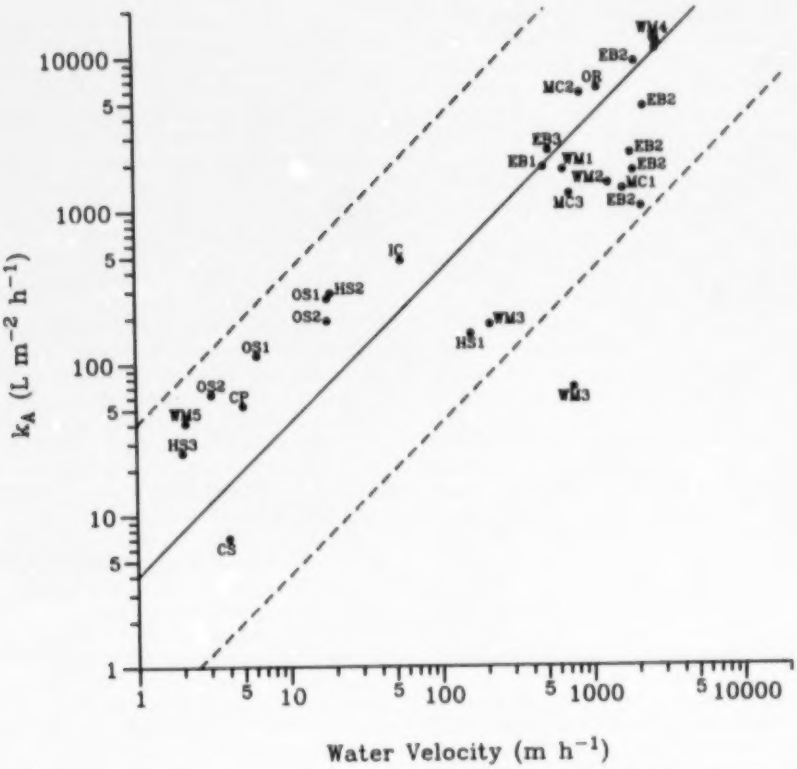

FIG. 2. Second-order substrate removal rate coefficients, k_A, based on colonized surface area, compared with a least-squares fit forced to a slope of direct proportionality. Two sites, WM3 and EB3, showed little or no substrate removal initially but exhibited active removal when tested later. The two initial data points were not used to calculate the best fit of direct proportionality. A 10-fold departure from the direct proportionality slope is indicated (-------). Site abbreviations: CP, Chalet Pond; CS, Corkscrew Swamp; EB1 to EB3, Elders Covered Bridge Park (EB1 and EB2, a granite-bottom stream; EB3, a sand-bottom area); HS1 to HS3, Harris Shoals Park; IC, Iron Creek; OS1 and OS2, Okefenokee Swamp; OR, Oconee River; MC1 to MC3, Mountain Creek (MC1, a gravel-bottom reach with little visible periphyton; MC2, a cobble-bottom reach with no visible periphyton; MC3, a cobble- and sand-bottom reach with no visible periphyton); WM1 to WM5, Watson Mill Bridge State Park.

composed of communities structured such that they can remove chemicals at near-maximum rates per unit of colonized surface area for substrates utilized via commonly occurring transport and transformation systems. The maximum removal rate may be determined by physicochemical characteristics of the substrate, and one or more organisms may be present in any diversely populated ecosystem that can remove the substrate at near-maximum rates. Consequently, although substrate removal rates may have varied considerably at the species level, community-level process rates were quite consistent among diverse communities.

We envision the application of our approach for field predictions as involving laboratory measurements of the substrate removal rates of test chemicals relative to one or more chemicals for which field data on k_A versus U are available. It is important that laboratory-derived rate coefficients be compared by using benchmark compounds, such as 2,4-DME, for which field-derived values are available. Otherwise, a complex mathematical model dealing with the effects of roughness of the streambed or other site-specific characteristics would have to be used (Gantzer, Ph.D. dissertation). Moreover, rate data for the benchmark chemical should be obtained from field systems similar to those systems to which the model is applied. A benchmark k_A determined in freshwater streams, for example, should probably not be used to predict substrate removal rates in wastewater treatment facilities.

Depending on water velocity, biofilm transformation rates of chemicals may vary somewhat when compared with transformation rates of a benchmark chemical. Relative transformation rate differences for 2,4-DBE and 2,4-DME were insignificant, however, in laboratory reactors with colonized Teflon strips. For example, the relative rate coefficient (mean $\pm$ SE) was 1.2 ± 0.05 with $n = 2$ at 240 m h^{-1} and 1.1 ± 0.00 with $n = 2$ at 1,700 m h^{-1}. The slope of k_A versus U for test chemicals, therefore, was assumed to be parallel to the slope of the benchmark chemical (Fig. 2). Whatever relative transformation rate coefficient value is used would be multiplied by the ordinal intercept of field data of the type illustrated in Fig. 2. This approach is appropriate as long as substrate removal rates are controlled by external mass transport. Theoretically, if water velocities are high enough or if intrinsic substrate removal rates are slow enough, the slope of k_A versus U will approach 0. In our studies of rapidly transformed 2,4-D esters, however, transformation rates continued to increase proportionally with U up to a velocity of 3,200 m h^{-1}. Moreover, the residence time may be too low in lotic systems for significant transfor-

mation of chemicals transformed too slowly to be externally mass transport limited. Predicting transformation rates of more slowly transformed hydrophilic chemicals may not, therefore, be important in lotic systems.

The relative rate coefficient for colonized Teflon strips, calculated by dividing k_1 values (mean ± SE) of 2,4-DBE by those of 2,4-DME, was 1.1 ± 0.04 with $n = 8$. With a relative rate coefficient of 1.1, half-lives for 2,4-DBE were predicted for field systems other than those used to develop k_A values for 2,4-DME. Predicted half-lives ranged 1,500-fold and were within about a factor of 3 or less of measured half-lives (Table 1). The system with the highest velocity, High Shoals, had a water velocity 1.4 times higher than that used to obtain the data in Fig. 2. The simple direct proportionality relationship in Fig. 2, therefore, was appropriate for predicting 2,4-DBE removal rates. The relative rate coefficient (mean ± SE) of 2,4-DBE to 2,4-DME, determined by adding the chemical mixture to the field sites shown in Table 1, was 0.96 ± 0.15 with $n = 5$, in agreement with our laboratory-derived value of 1.1 ± 0.04.

The flux of substrate into biofilms can be viewed as a function of three mechanisms: (i) external mass transport from the bulk liquid through a diffusion boundary layer to the biofilm surface, (ii) mass transport within the biofilm, and (iii) the intrinsic microbial transformation rate of the chemical by the microorganisms in the biofilm (Gantzer et al., in press). The effects of turbulence on mass transport-limited processes to and in the biofilm have typically been explained in terms of mass transport across diffusion boundaries (1, 3, 4, 6, 16, 19).

Differences in intrinsic substrate removal rates, however, have not been adequately explained and are currently subject to speculation. Substrate-enzyme reaction rates vary widely among substrates, but uptake rates probably also vary widely and may be slower than internal reaction rates.

Differences among substrates with respect to their affinities to transport proteins, hindrances to entering the cell wall, orientation of substrate molecules near the cell wall, or other physicochemical or steric effects may cause intrinsic differences in uptake rates. We prefer to view uptake rate differences as differences in uptake efficiencies related to mass transport of substrate molecules to uptake sites on cell walls. Substrate molecules that require an average of 10 encounters near uptake sites before uptake occurs, for example, would require 10 times the mass transport rate to equal the removal rates of substrate molecules that are taken up every time they approach uptake sites. The number and position of hydrogen atoms or other properties common to substrates could account for such effects.

The development of a mathematical model to predict chemical removal rates in the field necessitates ascertaining the respective contributions of suspended and attached organisms to the overall degradation process. Except in ecosystems unusually stressed by high nutrient inputs or nutrient depletion, we propose that similar proportions of suspended- and attached-organism activities exist among similar field conditions.

To test this hypothesis, we normalized k_1 for attached populations to a standard U and A/V. Under conditions of mass transport limitation, k_1 for attached organisms was directly proportional to U and to A/V. The normalized k_1 for each site of attached populations was compared with the corresponding independently determined k_1 for suspended populations. Values of k_1 for suspended populations were constant, regardless of water velocity. We had nine field areas in which we obtained k_1 for suspended organisms. When normalized to 0.01 m^2 of colonized surface area $liter^{-1}$ of water and 300 m h^{-1} bulk water velocity, the contribution of suspended organisms to the total k_1 was 0.11 ± 0.04%. The consistency of the relative contributions of suspended and attached communities, therefore, indicated that similar proportions of suspended and attached communities do indeed exist among similar field conditions. We now define the fraction of suspended-organism activity, f_s, for a specific ratio of colonized surface area to volume, by equation 2, where k_s and k_a are pseudo-first-order rate coefficients for the suspended and attached microbial activities, respectively.

$$f_s = k_s/(k_a + k_s) \quad (2)$$

We assumed that suspended-organism activities were evenly distributed throughout the water column; therefore, the total contribution of suspended organisms was assumed to be directly proportional to D. These relationships are expressed in equation 3, which incorporates our field-determined rate coefficients, k_{a1} and k_{s1}, normalized to a velocity, U_1, of 300 m h^{-1} and a depth, D_1, of 0.1 m.

$$f_s = (D/D_1)k_{s1}/[(U/U_1)k_{a1} + (D/D_1)k_{s1}] \quad (3)$$

Values of k_{a1} and k_{s1} (mean ± SE) were 46 ± 10 h^{-1} with $n = 12$, and 0.03 ± 0.003 h^{-1} with $n = 12$, respectively. Including these values yields equation 4.

$$f_s = D/(0.5U + D) \quad (4)$$

Because the fraction of suspended-organism compared with total-organism activity in swamps was similar to that in lotic systems, equation 4 was also applicable to swamps. Values for the fraction of suspended-organism activity for the two ponds were about 10-fold higher than for lotic systems.

Using equation 4 and our observed water velocities and depths, we calculated the relative contributions of attached

TABLE 1. Field data for 2,4-DBE removal rates

Site	U^b	D^c	Predicted $t_{1/2}$[d]	Measured $t_{1/2}$	Deviation[e]
High Shoals	3,200 ± 80	0.05 ± 0.010	0.0025	0.0045	1.8
Skidaway Creek	98 ± 13	0.04 ± 0.008	0.07	0.13	1.9
Corkscrew Pump Station	35 ± 4.2	0.04 ± 0.006	0.20	0.12	1.7
Orange River	11 ± 0.6	0.03 ± 0.005	0.50	0.26	1.9
Okefenokee Canal	3.3	0.08 ± 0.009	3.7	1.2	3.1

[a] Values represent mean ± SE whenever replicate measurements were made.
[b] Water velocity in meters $hour^{-1}$.
[c] Water depth in meters.
[d] Based on relative rate coefficient value of 1.1, which was calculated from laboratory-derived k_1 values of 2,4-DBE divided by k_1 values of 2,4-DME. Half-life ($t_{1/2}$) values in hours.
[e] Deviation of measured $t_{1/2}$ values greater or less than predicted $t_{1/2}$. A threefold deviation, for example, above or below a predicted $t_{1/2}$ equaled a deviation of 3.0.

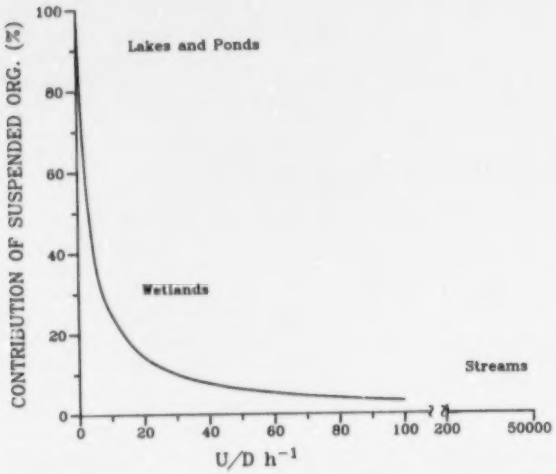

FIG. 3. Based on the relationship between the activities of attached and suspended organisms, and empirically derived coefficients, the relative contributions of attached and suspended communities can be predicted from the ratio of water velocity to depth of aquatic ecosystems. Their relative contributions in various types of ecosystems, such as lakes, rivers, wetlands, and streams, also can be deduced from this relationship.

and suspended communities in natural ecosystems (Fig. 3). The activities of attached populations in deep lentic ecosystems were insignificant fractions of total organism (suspended plus attached) activities (Fig. 3). Attached populations in lakes or ponds, however, would certainly be significant for processes such as anaerobic transformations that may be exclusively carried out in association with the attached organisms. Also, the relative activities of suspended organisms in streams or shallow rivers (approximately 1 m in depth or less) are expected to be insignificant. This conclusion agrees with that of Gantzer (Ph.D. dissertation), who developed a more rigorous equation that included measurements of suspended biomass, substrate flux into the biofilm, and a ratio of colonized surface area to stream width.

Moreover, Fig. 3 suggests that suspended communities represent only about 20 to 40% of the total activity occurring in wetlands. Our field experiments, however, did not include epiphytic communities. Suspended microorganisms in wetlands, therefore, may account for even less of the total microbial activity than indicated in Fig. 3. The contributions of attached communities in deep rivers is probably much less than that indicated by our simple proposed relationship to U/D. For example, rapidly utilized substrates could be substantially removed by suspended organisms before they are even transported to colonized subsurfaces. Also, U is not a good indicator of benthic turbulence in deep rivers (20).

On the basis of our relationship of suspended- and attached-organism activities to U/D, wetlands appear potentially to be chemical sinks. Deep lentic ecosystems have large water columns of suspended organisms to rapidly degrade such chemicals. Shallow lotic ecosystems have sufficient water velocities to facilitate rapid removal of mass transport-limited substrates by attached populations. Wetlands, however, have both shallow water columns and low water velocities, indicating that wetlands are relatively inefficient with regard to substrate removal rates. Our simple view of dissolved substrate removal rates being largely determined by water depth, velocity, and colonized surface area suggests that wetlands can increase their efficiency for substrate removal only by increasing colonized surface area. The typical inundation of wetlands by macrophytes, which also support a large colonized surface area of epiphytic microflora, may allow wetlands to successfully compete with other ecosystems for efficient substrate removal.

Because transformations of the 2,4-D esters we studied are carried out by almost all heterotrophic microorganisms (9, 13), we expect Fig. 3 to be representative, in general, of substrate removal rates of such organisms. Therefore, even if our empirically determined coefficients in equation 4 are relatively imprecise, the general shape of and conclusions drawn from Fig. 3 should be valid. Consequently, equation 4 may have very broad application in more general mathematical models that attempt to account for pollutant degradation by both attached and suspended microorganisms.

We have presented a relatively simple approach that we expect to have broad application for estimating substrate removal rates at field sites. The predictive accuracy of our present model depends upon the extent to which systems are externally mass transport limited, the physicochemical similarity of test chemicals to the benchmark chemical, and the similarity of field systems to those systems in which k_A was determined for the benchmark chemical. For an improved predictive capability, our methods may be tailored to various applications. Relating substrate removal rate coefficients to shear velocities, which account for streambed roughness, for example, may be useful. A standard procedure for laboratory measurements of relative rate coefficients, which takes into consideration the effects of physical biofilm characteristics, diffusion coefficients, and mass-transfer coefficients, would also be helpful.

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